REMARKS

The specification is amended above to insert a reference to related cases.

Several obvious typographical errors are corrected on pages 3, 6, 7 and 11.

The obvious incorrect translation/typographical error "NADPH cytochrome P450" has been amended to read correctly --cytochrome P450-- on page 4, 8 and 9 of the specification.

SEQ ID NO's have been added in the appropriate location on page 24.

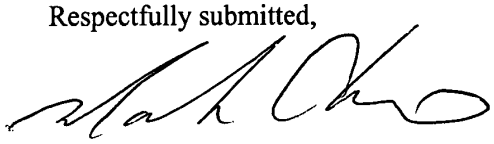
Claims 3 and 4 have been amended to correct an obvious translation/typographical error. One of ordinary skill in the art would know and understand that there is no enzyme "NADPH cytochrome P450", only "cytochrome P450" or "NADPH cytochrome P450 reductase".

Claims 8, and 10-11 are amended to better format the international claims for accepted US Patent practice.

No amendment of inventorship is necessitated by these amendments.

Early allowance of the claims is requested. Should the Examiner believe that a conference with applicants' attorney would advance prosecution of this application, the Examiner is respectfully invited to call applicants' attorney.

Respectfully submitted,



Dated: March 12, 2003

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Mark-up of the Claims Showing Changes Made in the Specification

Page 3, 1st paragraph.

-- problematic in that not only the stability of cultured cell properties is considerably impeded due to a lack of the qualitative stability of the serum but also the stable, accurate and inexpensive use of established cells is considerably hampered due to the very high price of the serum. Accordingly, proliferation of an established immortalized cell culture in a serum-free medium, while stably retaining its **characters**, would be industrially very beneficial.--

On page 4, 3rd and 4th paragraphs.

--(3) the cell culture according to the above item (1) above wherein the enzyme is NADPH cytochrome P450 reductase, ~~NADPH~~ cytochrome P450, flavin monooxygenase, epoxy hydratase, glucurosyl transferase, sulfotransferase or glutathione S-transferase,

(4) the cell culture according to the above item (3) above wherein the ~~NADPH~~ cytochrome P450 is CYP1A1, CYP1A2 or CYP3A,--

Page 6, 6th paragraph

-- The human normal hepatocytes (preferably human normal hepatic parenchymal cells) used can be separated from normal tissue of human adults, human fetuses, etc. (preferably human fetuses) by a well-established method known as collagenase perfusion. What **are** ~~is~~ called primary cultured cells thus obtained are immortalized in accordance with various commonly known methods etc. Specifically, there may be mentioned a method focusing on the permanent proliferation of tissue which has cancerated wherein individual normal cells are immortalized by transformation with an oncogene introduced therein. Immortalized cell cultures thus established include, for example, subcultures of transformants of animal cells as obtained by introducing an oncogene, such as ras or c-myc, or an oncogene of a DNA type tumor virus, such as adenovirus EIA, SV (simian virus) --

Page 7, 1st paragraph

40 virus, or human papilloma virus (HPV16), or a tumor antigen (T antigen) gene thereof (E. Ponet et al., Proc. Natl. Acad. Sci., USA, 82, 8503 (1985)). Preferably, the method based on introduction of the T antigen gene of SV40 origin, a modification thereof, or the like can be used (M. Miyazaki et al., Experimental Cell Research, 206, 27-35 (1993)). To culture (subculture) these immortalized hepatocytes, there may be used commonly known culturing methods using known media [e.g., complete synthetic media (preferably serum-free complete synthetic media (e.g., ASF104 medium, Ajinomoto), MEM medium containing about 5 to about 20% fetal bovine serum [Science, Vol. 122, 501 (1952)], DMEM medium [Virology, Vol. 8, 396 (1959)], RPMI 1640 medium [The Journal of the American Medical Association, Vol. 199, 519 (1967)], Williams' medium (Nissui Pharmaceutical), 199 medium [Proceedings of the Society for the Biological Medicine, Vol. 73, 1 (1950)]. Complete synthetic media [serum-free complete synthetic media (e.g., ASF104 medium, Ajinomoto)] etc. are particularly preferred. The pH is **preferable** about 7 to about 7.2. Cultivation is normally carried out at about 37°.

Page 7, 3rd paragraph

-- From among the immortalized hepatocytes thus obtained, those retaining metabolic **characteristics** specific to the liver, more specifically enzyme activity, enzymes, gene expression and gene expression induction associated with the metabolism of xenobiotics, are selected.--

On page 8, last paragraph to page 9 1st paragraph.

Enzymes involved in the liver-specific metabolism of xenobiotics include, for example, NADPH cytochrome P450 reductase, ~~NADPH~~ cytochrome P450, flavin monooxygenase, epoxy hydratase, glucurosyl transferase, sulfotransferase, and glutathione S-transferase. Of these enzymes, ~~NADPH~~ cytochrome P450 represents the class of enzymes most important from the viewpoint of distribution and functions in the metabolism of xenobiotics. ~~NADPH~~ cytochrome P450 is a generic name for a large number of enzymic proteins; CYP1A1, CYP1A2, CYP2A6,

[redacted]

CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A (specifically CYP3A4, CYP3A5, CYP3A7 etc.), CYP2D6 etc. are known members of the ~~NADPH~~ cytochrome P450 class involved in the metabolism of xenobiotics in the human liver, with CYP1A1, CYP1A2, CYP3A etc. preferably used for the immortalized hepatocyte culture of the present invention. In addition, the functions of ~~NADPH~~ cytochrome P450 are also generically called the mixed function oxidation (MFO) and are detected as ethoxyresorufine dealkylation activity, benzyloxyresorufine dealkylation activity, pentoxylresorufine dealkylation activity, methoxyresorufine dealkylation activity etc. Furthermore, the presence of NADPH cytochrome P450 reductase is essential to the expression of the MFO functions of the ~~NADPH~~ cytochrome P450 protein; this enzyme can also be classified as an enzyme which metabolizes xenobiotics.

Page 11, 3rd paragraph.

-- A pharmaceutical containing a compound obtained by said screening method or a salt thereof can be produced by a commonly known production method or a method based thereon. The preparations thus obtained can be used **with ~~to~~**, for example, humans or mammals (e.g., rats, mice, guinea pigs, rabbits, sheep, swine, bovines, horses, cats, dogs, monkeys) because they are safe and of low toxicity. --

Page 24, 1st paragraph

-- respective types of cytochrome P450 available from the Gene Bank database. The accession numbers at the Gene Bank are K03191 for CYP1A1, M55053 for CYP1A2, J02625 for CYP2E1, J04449 for CYP3A4, J04813 for CYP3A5, and D00408 for CYP3A7. The individual primers used were

5'- ATGCTTTTCC CAATCTCCAT GTGC **(SEQ ID NO:1)** and

5'- TTCAGGTCCT TGAAGGCATT CAGG **(SEQ ID NO:2)** for CYP1A1,

5'- GGAAGAACCC GCACCTGGCA CTGT **(SEQ ID NO:3)** and

5'- AAACAGCATC ATCTTCTCAC TCAA **(SEQ ID NO:4)** for CYP1A2 and

5'- ATGGCTCTCA TCCCAGACTT G **(SEQ ID NO:5)** and

5'- GGAAAGACTG TTATTGAGAG A **(SEQ ID NO:6)** for CYP3A. --

CLAIMS AFTER AMENDMENT

1. (Original) An immortalized hepatocyte cell culture of human normal cell origin retaining an enzyme activity involved in the metabolism of xenobiotics in the liver or the capability of expressing a gene encoding an enzyme involved in the metabolism of xenobiotics in the liver.

2. (Original) The cell culture according to Claim 1 wherein the enzyme activity is NADPH cytochrome P450 reductase activity, glucuronosyl transferase activity, ethoxyresorufine dealkylation activity, benzyloxyresorufine dealkylation activity, pentoxylresorufine dealkylation activity, methoxyresorufine dealkylation activity, flavin monooxygenase activity, epoxy hydratase activity, sulfotransferase activity or glutathione S-transferase activity.

3. (AMENDED) The cell culture according to Claim 1 wherein the enzyme is NADPH cytochrome P450 reductase, cytochrome P450, flavin monooxygenase, epoxy hydratase, glucurosyl transferase, sulfotransferase or glutathione S-transferase.

4. (AMENDED) The cell culture according to Claim 3 wherein the cytochrome P450 is CYP1A1, CYP1A2 or CYPA3.

5. (Original) The cell culture according to Claim 1 wherein the cell culture is FERM BP-6328.

6. (Original) A method of producing the cell culture according to Claim 1, characterized by introduction of the T antigen gene of SV (simian virus) 40 origin into human normal hepatocytes.

7 (Original) The production method according to Claim 6 wherein the human normal hepatocytes are hepatocytes of human fetal origin.

8. (Amended) A screening method for a compound or a salt thereof 1) which inhibits or promotes an enzyme activity involved in the metabolism of xenobiotics in the liver or 2)

which inhibits or promotes the expression of a gene encoding an enzyme involved in the metabolism of xenobiotics in the liver, said method characterized by the use of the cell culture according to Claim 1 in a screening assay comprising contacting said cell culture with a compound to be tested and detecting enzyme activity or gene expression in said cell culture.

9. (Original) A compound or a salt thereof 1) which inhibits or promotes an enzyme activity involved in the metabolism of xenobiotics in the liver or 2) which inhibits or promotes the expression of a gene encoding an enzyme involved in the metabolism of xenobiotics in the liver, obtained by using the screening method according to Claim 8.

10. (AMENDED) An analytical method for assaying (a) enzymes involved in the metabolism of xenobiotics and/or endogenous substrates, (b) metabolic pathways for xenobiotics and/or endogenous substrates, (c) chemical structures of metabolites of xenobiotics and/or endogenous substrates, (d) inhibition of enzymes which metabolize xenobiotics and/or endogenous substrates, (e) promotion of the activity of enzymes which metabolize xenobiotics and/or endogenous substrates, (f) cytotoxicity due to the metabolism xenobiotics and/or endogenous substrates, (g) genotoxicity due to the metabolism xenobiotics and/or endogenous substrates, (h) carcinogenicity due to the metabolism xenobiotics and/or endogenous substrates, (i) mutagenicity due to the metabolism xenobiotics and/or endogenous substrates, (j) hepatotoxicity due to the metabolism xenobiotics and/or endogenous substrates, or (k) hepatic action of xenobiotics and/or endogenous substrates, characterized by the use of the cell culture according to Claim 1 in an assay comprising contacting said cell culture with a test subject, then assaying for the resultant effect on the cell culture.

11. (Amended) A method of preparing metabolites of xenobiotics and/or endogenous substrates, characterized by the use of the cell culture according to Claim 1 comprising maintaining said cell culture in the presence of xenobiotics and/or endogenous substrates for sufficient time under conditions suitable for the production of metabolites of said xenobiotics and/or endogenous substrates, and producing said metabolites.

[redacted]